

Neuroscience

P1
4-HYDROXY-2-NONENAL MODIFIES NEUROFILAMENT PROTEIN, IMPAIRS AXON OUTGROWTH, CAUSES ACCUMULATION OF MITOCHONDRIA IN ABERRANT AXONAL STRUCTURES AND MIMICS THE EFFECT OF DIABETES IN CULTURED ADULT SENSORY NEURONS.

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Introduction: Protein adducts of the lipid peroxidation product 4-hydroxy-2-nonenal (4-HNE) have been associated with disease states involving oxidative stress. Modification of proteins by 4-HNE in peripheral nerve tissue occurs in diabetic sensory neuropathy, however, the target proteins and impact on nerve function are unknown. We, therefore, tested the ability of 4-HNE to induce amino acid adduct formation on axonal cytoskeletal proteins and determined if such modifications were associated with aberrant axon morphology, mitochondrial accumulation, and suboptimal axon outgrowth in cultured adult sensory neurons. The impact of type 1 diabetes on these processes was also investigated. Methods: Adult rat dorsal root ganglion (DRG) sensory neurons were cultured in defined F12-media supplemented with neurotrophic factors (NTFs; 1 nM insulin, 1ng/ml NGF, 10ng/ml GDNF and 10ng/ml NT-3) and treated with 4-HNE concentrations ranging from 1.0µM to 10 µM. Cell survival, axonal morphology and level of axonal regeneration were assessed at 24 hours in culture. Western blot and immunofluorescent staining were utilized to detect protein adduct formation by 4-HNE (anti-4-HNE, Alexis biochemicals) and phosphorylation levels of neurofilament H protein (antibody SMI31, Covance). Also, a mitochondrial specific dye (1.0 µM Mitotracker red CMXRos; Invitrogen) was utilized to detect the presence of mitochondrial accumulation in the aberrant structures along the axons. Results: 4-HNE induced formation of amino acid adducts on neurofilament H protein and impaired axon regeneration by approximately 50% (ED50 3µM) whilst having no effect on neuronal survival. 4-HNE initiated formation of aberrant axonal structures and caused the accumulation of mitochondria in these structures, which mimicked those seen in axons of neurons under diabetic conditions (in animal models and humans). The formation of protein adducts also led to diminished levels of phosphorylation of neurofilament H protein. Sensory neurons from 3 month streptozotocin-diabetic rats showed abnormal axonal swellings which were filled with 4-HNE protein adducts and impaired levels of axon outgrowth; control neurons exhibited negative staining for 4-HNE in axons. Conclusion(s): This study demonstrates that 4-HNE

induces amino acid adduct formation on neurofilament H protein, causes mitochondria accumulation, and this modification is associated with impairment of axonal regeneration. The results show that 4-HNE might be an important link between oxidative stress triggered lipid peroxidation and subsequent modification of key neuronal cytoskeletal and mitochondrial proteins in diabetic sensory neuropathy. It is proposed that 4-HNE mediates abnormalities in neurofilament and mitochondrial function, and possibly other cytoskeletal proteins, and may cause distal axon degeneration through suboptimal mitochondrial motility and localization.

P3
IDENTIFICATION OF NOVEL DRUG CANDIDATES FOR TREATMENT OF NEUROLOGICAL COMPLICATIONS ASSOCIATED WITH DIABETES.

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Objectives. The symmetrical sensory polyneuropathy exhibited by persons with type 1 and type 2 diabetes is the most common form of peripheral neuropathy and has an increasingly detrimental impact on human disease and associated health costs - there is no effective treatment. Though etiology remains poorly understood a dying back of distal axons from the peripheral target and a failure of axonal sprouting and/or regeneration are key features of this neurodegenerative disease. The heterogeneous nature of this neurodegenerative disease has confounded attempts based upon hypothesis-driven research to generate an effective therapy. Therefore, we elected to test the therapeutic potential of a group of pre-screened small molecule FDA-approved compounds for efficacy in rodent models of type 1 diabetes exhibiting diabetic sensory neuropathy. As part of a Juvenile Diabetes Research Foundation (JDRF)-funded drug screen we assessed 500 compounds from an NINDS drug library for the ability to significantly raise axon outgrowth in an in vitro assay consisting of cultured adult rat dorsal root ganglia (DRG) sensory neurons. Materials and methods. The assay comprised cultured adult rat sensory neurons and the ability of drugs to enhance total axonal outgrowth by at least 2-fold (P<0.05). The rationale was that axon outgrowth in this in vitro model was homologous to axon plasticity, growth and regeneration occurring in the epidermis of the skin. In diabetic sensory neuropathy the distal dying-back of axons is a key pathological feature and to this date not a single compound has proved efficacious in reversing axonal loss in the epidermis (in animal models or humans). Adult neurons were isolated from age matched control animals, or 3 month streptozotocin (STZ) diabetic rats (type 1 model) or 4 month Zucker diabetic fatty (ZDF) rats (type 2 model). DRG sensory neurons were cultured in Hams F-12 media under defined conditions in a sub-saturating cocktail of

neurotrophic factors and exposed to drugs for 24 hr before being assessed for total levels of axon outgrowth and cell survival using a morphometric approach (a Weibel grid approach to be specific). Results. Data have been generated from primary, secondary and tertiary screening of 18 promising drugs that increased axon outgrowth by 2-fold ($P < 0.05$) in normal neurons. In the secondary and tertiary screens these 18 compounds were screened against adult cultures derived from STZ-diabetic rats and ZDF rats. In the STZ-diabetic rat screen guaifenesin, guanethidine sulphate and pirenzepine HCl elevated axonal outgrowth by at least 2-fold at certain doses. Interestingly, the same 3 compounds were effective in the cultures derived from ZDF rats. Therefore, at this stage the most promising compounds we have identified for further testing are guaifenesin, pirenzepine HCl and guanethidine sulphate, in addition ethopropazine HCl was effective against cultures from ZDF rats. Conclusions. The four promising compounds noted above are now being exposed to medicinal chemistry to generate novel compounds for further in vitro and in vivo testing.

P4
PRELIMINARY ANALYSIS OF PLAQUE FORMATION ASSOCIATED WITH ALZHEIMER'S DISEASE IN TRANSGENIC MICE: A COMPARATIVE LONGITUDINAL STUDY.

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Alzheimer's Disease (AD) is predominated by neuronal loss resulting from the deposition of extracellular senile plaques composed of β -amyloid peptides. Unfortunately due to the difficulty associated with identifying plaques in vivo, confirmation of the disease is only certain after post mortem analysis on the brain is completed. As such diagnostics of AD rely heavily on cognitive tests to assess judgment, decision-making and memory in an effort to rule out other types of dementia. Much research is currently focused on developing non-invasive techniques for evaluating AD in animal models. We developed a comparative MRI study where T2-weighted images were acquired from both an AD transgenic mouse and an aged matched wild type control littermate (7 and 11 months respectively). The basic measurement parameters included: a multi-slice, multi-echo spin-echo pulse sequence (MSME), echo time = 20 ms, repetition time = 1700ms, averages = 2, field of view = 26 x 26 mm and image matrix = 256x256. This provided an effective in-plane resolution of approximately 100x100 μm^2 . Following MR image acquisition the animals were euthanized and brain slices were analyzed by Congo red staining (gold standard). Data acquired by these methods will be compared to determine the feasibility of the clinical application of MR technology in studying AD in vivo.

P5
METABOLIC REGULATION OF NEURONAL EXCITABILITY AS A FACTOR IN AGE-RELATED LEARNING IMPAIRMENT.

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Age-dependent impairment in learning and memory functions occurs throughout the animal kingdom. Although cell death contributes to age-related cognitive impairment in pathological forms of aging, learning and memory deficiencies develop with age even without substantial cell death. The molecular and cellular basis of this biological aging process is not well understood but seems to involve a decline in the aging brain's capacity for experience-dependent plasticity. To aid in resolving this issue, we use simple invertebrate neurobiological correlates of learning and memory that allow us to directly study the cellular and molecular causes of age-associated learning and memory deficiencies down to the resolution of single neurons and identified neural networks. Our latest results obtained with this model system indicate that age does not affect the acquisition of (appetitive) memory but that retention and/or consolidation of long-term memory become progressively impaired with advancing age. This phenomenon correlates with declining electrophysiological excitability in key neurons controlling the behavior involved in this learning paradigm. Our work implicates oxidative stress and redox modulation of (transient) voltage-gated K^+ channel functions as one of the key molecular factors underlying this decline in excitability. Thus, our data points towards a role for metabolic regulation of neuronal membrane excitability in the decline of plastic capacity of the biologically aging brain.

P6
HIGH GLUCOSE CONCENTRATION ELEVATES OXIDATIVE STRESS IN ADULT SENSORY NEURONS FROM TYPE 1 DIABETIC RATS.

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Objectives. Sensory neuropathy, which is induced by diabetes in humans and in experimental animal models, can present such complications as sensory loss, foot ulceration and infection. Lower limb gangrene and amputation can follow. In diabetes high blood glucose concentration and lack of insulin signaling are key factors in induction of abnormalities in polyol pathway flux, protein glycation and neurotrophic support in the peripheral nervous system (PNS). Combination of those factors can enhance oxidative stress and trigger distal nerve damage in PNS. Elevated generation of reactive oxygen species (ROS) and mitochondrial dysfunction has been proposed as a one of the critical factors in nerve degeneration in diabetes. We tested the hypothesis

that sensory neurons exposed to long term type 1 diabetes in vivo would exhibit enhanced ROS and oxidative stress in perikarya and axons and tested if this stress was associated with impaired regenerative axon outgrowth in cell culture. Methods. Lumbar dorsal root ganglia (DRG, L1-L6) sensory neurons were isolated from age matched normal and 3-month streptozotocin (STZ)-diabetic rats and cultured in defined F-12 media supplemented with N2 additives under either normal (10mM) or high (25-50mM) glucose concentrations. Levels of cell survival and axon outgrowth were assessed. Also, level of ROS was analyzed in perikarya and axons using dihydrorhodamine 123 and 5-(and-6)-chloromethyl-2', 7'-dichlorohydrofluorescein diacetate (CM-H2DCFDA) using real time video confocal microscopy. In addition, mitochondrial preparations from DRG were assessed for rates of electron transport by measuring rates of oxygen consumption (Clarke electrode). Additionally, immunofluorescent staining was used to detect expression of phosphorylated neurofilament H (antibody SMI31), neuron-specific beta-tubulin III and amino acid adducts of 4-hydroxy-2-nonenal (4-HNE). Furthermore, MitoFluor green dye was used for colocalization of mitochondria in the axons in formalin-fixed cultures of DRG neurons. Results. DRG sensory neurons isolated from diabetic rats exhibited a 2-fold ($P<0.001$) elevation of ROS levels in axons after 24 hrs of culture compared with control. However, the perikarya exhibited no change in ROS levels. At 4 days in vitro levels of neurotrophin-induced axonal growth were significantly reduced by 2.3-fold ($P<0.001$) in diabetic cultures compared with control. Acute and longer term (24 hr) treatment with 1mM N-acetyl-cysteine significantly lowered axonal ROS levels and prevented the deficit in axonal outgrowth in diabetic neurons. Mitochondrial preparations from DRG of diabetic rats demonstrated enhanced rates ($P<0.05$) of coupled and uncoupled electron transfer. We also discovered that axonal morphology of diabetic neurons was abnormal with appearance of dystrophic swellings that were filled with neurofilament, mitochondria and amino acid adducts of 4-hydroxy-2-nonenal. Conclusions. We show for the first time that DRG sensory neurons with a history of diabetes express high levels of ROS in their axons but not in the perikarya. Oxidative stress is limited to the axonal compartment in diabetic neurons, is associated with elevated mitochondrial electron transport and results in impaired axon outgrowth.

Nutrition

P8

PROTECTIVE EFFECTS OF SASKATOON BERRIES, GREEN TEA EXTRACT AND BROCCOLI SPROUTS AGAINST MYOCARDIAL ISCHEMIA-REPERFUSION INJURY.

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Source of Research Funds: Canadian Institutes of Health Research.

Ischemia-reperfusion (IR) injury occurs following clinical events such as heart surgeries. Dietary interventions may be useful strategies to suppress the consequences of IR. The objective was to examine the extent to which flavonoid-rich foods can reduce oxidative stress and cell death induced by myocardial IR in rats, and the possible mechanisms involved. Catechin-rich green tea extract and anthocyanin-rich saskatoon berries as sources of flavonoids and broccoli sprouts as a source of phase 2 protein inducers were studied. Rats in 5 groups were fed either regular diet (control and sham groups), or diets mixed with either freeze-dried berries (5% of diet), tea extract (0.25% of diet), or dried broccoli sprouts (2% of diet) for 10 days before IR. Ischemia was induced globally for 20 min in excised hearts mounted on a Langendorff apparatus followed by reperfusion for 2 h. Cell death and oxidative stress measurements were performed to assess the IR injury. Feeding broccoli sprouts gave good protection against myocardial cell death by either necrosis or apoptosis (78-86%, $p<0.05$). Feeding green tea extract also reduced apoptosis by 54% ($p<0.05$). Broccoli sprouts and berries decreased myocardial lipid peroxidation by 116 and 73%, respectively ($p<0.05$). Broccoli sprouts was the only intervention that improved the coronary flow rate. Green tea was the only intervention which prevented (78%) a drop in glutamate cysteine ligase activity after IR. None of the interventions induced heme oxygenase-1. Overall the results showed that dietary interventions with flavonoid-rich foods protected hearts against myocardial IR injury. The best protection was exerted by broccoli sprouts, but apparently through mechanisms other than induction of the phase 2 enzymes, glutamate cysteine ligase and heme oxygenase-1.

ABSTRACTS POSTER PRESENTATIONS, JUNE 18-19, 2008

P9

NORMAL AND PHYSIOCHEMICALLY MODIFIED WHEAT BRAN MAY REDUCE FAT MASS AND INCREASE FAT OXIDATION IN A HAMSTER MODEL.

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Wheat bran is a major source of dietary fibre in North America. Physiochemical modifications of wheat bran result in novel fibre preparations that may be beneficial in reducing chronic disease risk factors. The present study examined the effect of NovoBran, a physicochemically modified wheat bran with increased antioxidant capacity, on circulating lipid concentrations, energy expenditure and body composition. Using a hypercholesterolemic hamster model, 60 Syrian golden males were randomized into 4 groups and fed a basal control diet or the control diet supplemented with 2% plant sterol (positive control), 10 % normal wheat bran or 10% NovoBran, at the expense of corn starch. No differences were observed for circulating lipids or glucose between the treatment and control animals. However, correlation analysis revealed that consumption of normal wheat bran was negatively associated with blood glucose ($r = -0.590$; $P = 0.026$), CO₂ production ($r = -0.537$; $P = 0.048$) and positively correlated with total lean body mass ($r = 0.538$; $P = 0.030$). NovoBran consumption was negatively correlated with O₂ consumption ($r = -0.711$; $P = 0.006$) and final body weights in NovoBran fed animals were positively correlated with total lean body mass ($r = 0.829$; $P = 0.0002$), indicating high metabolic rates. Lastly, a negative correlation between the consumption of total wheat bran (combined normal bran and NovoBran animals) and total fat mass ($r = -0.509$; $P = 0.006$) was observed. In conclusion, these data suggest that the consumption of wheat bran and NovoBran may be effective dietary approaches to reducing body fat and adiposity.

P10

SAFETY, BUT LACK OF EFFICACY, OF MILK ENRICHED WITH CONJUGATED LINOLEIC ACID IN OVERWEIGHT, HYPERLIPIDEMIC ADULTS.

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To assess efficacy and safety of conjugated linoleic acid as a weight loss supplement in humans, a single blind, crossover trial was conducted wherein 15 moderately overweight (BMI=25-30 kg/m²), hyperlipidemic (LDL-C \geq 2.5 mmol/L) adults were randomized to receive (i) milk naturally enriched with 4.2 % *c*9, *t*11 CLA (NCLA), (ii) milk with added 4.2 % of a synthetic mixture of *c*9, *t*11 and *t*10, *c*12 isomers (SCLA), or (iii) regular milk (CONT) during 3 consecutive 8-week phases, each separated by a 4-week wash-out. Body composition using MRI, plasma lipid profile, inflammation markers C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF- α), and indices of liver function namely, plasma alanine transaminase (ALT) and total bilirubin (TBIL) were determined at baseline and endpoint of each treatment phase. Results indicate that there were no significant differences in the effects of the three milk treatments, either at phase-end or over time, on body weight and composition. With regards to plasma lipids, compared to control, LDL-C concentrations following CLA treatments remained unaltered (CONT: 3.37 ± 1.07 , NCLA: 3.32 ± 0.77 and SCLA: 3.53 ± 0.94 mmol/L). Similar results were obtained for plasma concentrations of HDL-C, TG and total cholesterol. In addition, no significant differences were observed between effects on safety parameters tested, including CRP (4.71 ± 1.01 mg/L, 4.79 ± 0.98 mg/L, 4.21 ± 1.06 mg/L), following 8 weeks of treatment with CONT, NCLA and SCLA respectively. These results suggest that under the present conditions, milk enriched either naturally or synthetically with CLA might not be a suitable weight loss agent in humans. However, CLA consumption does not exert detrimental effects.

**P11
WHOLE AND FRACTIONATED PEA FLOUR FAILS TO ELICIT CHANGES IN LIPID LEVELS AND BODY COMPOSITION, BUT ALTERS CAECUM MICROBIAL POPULATIONS IN GOLDEN SYRIAN HAMSTERS.**

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Few studies have compared the effects of whole and fractionated pea flours on risk factors for cardiovascular disease. The purpose of this study was to evaluate the effect of yellow pea fractions on circulating lipid levels, body composition, and caecum bacterial populations in the golden Syrian hamster model. Sixty golden Syrian hamsters were randomized to receive diets containing whole pea flour (10% w/w), pea hull flour (10% w/w), plant sterols (2% w/w) (positive control), or control for 28 days. On day 28, fat body mass (FBM) and lean body mass (LBM) were determined by dual X-ray absorptiometry (DXA). Blood and caecum samples were taken for lipid and gut microbiology analyses. Microbial communities were evaluated using terminal restriction length polymorphism analysis (TRFLP) using universal primers specific to 16s rDNA prokaryotic sequences. The terminal restriction fragments (TRF) were putatively assigned to phylogenetic groups using a bioinformatic assignment tool. The results indicated that, compared to control, whole and fractionated pea flour had no effect on TC, LDL-C, HDL-C and TG. Caecum analysis indicated that hamsters fed diets containing whole pea flour had a 4, 5 and 7 – fold increase in orders Bacteroidetes, Lactobacillales and Firmicutes, respectively. Pea hull flour caused a 2-fold reduction in Lactobacillales TRF prevalence. Furthermore, animals receiving pea hull flour had no TRF's belonging to the Bacteroidetes order and there was only a 1.5-fold increase in Clostridia compared to controls. The results indicate that whole and fractionated yellow pea flours have no effect on circulating lipid levels, FBM and LBM in the golden Syrian hamster. However, whole peas can elicit substantial changes in certain gut microbial populations, suggesting that whole peas possess prebiotic effects.

**P12
PHYTOSTEROL CONSUMPTION DECREASES HEPATIC NUCLEAR SREBP2 EXPRESSION IN HAMSTERS FED AN ATHEROSCLEROTIC DIET.**

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Although in vitro investigations suggest that phytosterols and cholesterol share similar molecular targets, data regarding the regulation of hepatic sterol regulatory element binding protein 2 (SREBP2) processing in response to dietary phytosterol consumption are lacking. Therefore, the objective of this investigation was to characterize the molecular events involved in the hypocholesterolemic response to phytosterol consumption at the hepatic level. Thirty hypercholesterolemic Syrian golden male hamsters were fed a basal control diet or the control diet supplemented with 2% phytosterols (w/w) for 28 days. Consumption of phytosterols reduced ($P < 0.002$) plasma total cholesterol (7.8 ± 0.46 vs. 5.3 ± 0.44 mmol/L), HDL-cholesterol (3.8 ± 0.09 vs. 3.2 ± 0.15 mmol/L), and non-HDL cholesterol (3.8 ± 0.35 vs. 2.1 ± 0.35 mmol/L) in comparison with the control group. In comparison with the control group, protein expression of the nuclear active form of hepatic SREBP2 was reduced (2.4 fold, $P < 0.005$) in response to the consumption of phytosterols. The results of this study suggest that regulation of hepatic cholesterol metabolism through a reduction in nuclear active SREBP2 expression may contribute to the hypocholesterolemic effects associated with phytosterol consumption. Future work will characterize the molecular signals involved in phytosterol-mediated SREBP2 expression and its subsequent effects on endogenous cholesterol synthesis and peripheral cholesterol scavenging.

P13
THERAPEUTIC EFFECT OF A DIET WITH SYNBIOTIC FLAX IN ADULTS.

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Synbiotic Flax is a novel product of complex processing applied to flax seed, resulting in the enhancement of the properties up to synbiotic formula being confirmed by lab testing. For the purpose of focusing planned future clinical trials, a preliminary pilot study investigating the effect of synbiotic formula including diet was performed in adults with 60% of the participants aged over 45 years. Each of four doctors was assigned 5 patients, for 6-8 months, daily consuming 15-30g of synbiotic formula as addition to their regular diet. The following results are based on the monitoring of venous blood chemistries, and a medical, sociological, psychological inventory of the patient's life history. Synbiotic Flax vastly improved quality of life for all participants having broad range of health issues at the beginning of the study. Up to 72% improvements were achieved in many age-related conditions including imbalance in intestinal microflora and digestion; chronic back, shoulders and joint pain; mobility; migraine; vision problems; immune system, uric acid profile; rheumatoid arthritis; gout; blood sugar level; fibromyalgia; hypertension; cognitive ability; ability to think and solve problems, memory; PSA level; overall blood profiles including seed rate profile, lipid profiles; physical and psychological profiles; weight control (loss up to 40lbs in 8 month); chronic condition of recurring colds and flu; hair and skin condition; toxicity; energy level; bowel movement; stress level. We realize that this was a very unusual preliminary pilot study due to the fact that there are so many variables and factors to check medically, socially and psychologically. Broad range of improvements in the components of the quality of life including age-related conditions seen in the patients during the pilot study suggest possibility of very complex therapeutic effect of Synbiotic Flax should be considered when planning further clinical trials.

P15
EFFECT OF CREATINE SUPPLEMENTATION AND RESISTANCE-EXERCISE TRAINING ON MUSCLE IGF-I.

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The purpose of this study was to compare changes in muscle insulin-like growth factor-I (IGF-I) content resulting from resistance-exercise training (RET) and creatine supplementation (CR). Male (n=24) and female (n=18) subjects with minimal resistance-exercise training experience (\leq 1year) volunteered for the study. Subjects were randomly assigned in blocks (gender) to supplement with creatine (CR: 0.25 g·kg⁻¹lean tissue mass for 7 days; 0.06 g·kg⁻¹ lean tissue mass for 49 days; n=22; 12 males, 10 female) or isocaloric placebo (PL: n=20; 12 male, 8 female) and engage in a whole-body RET program for 8 wks. Eighteen subjects were classified as vegetarian (lacto-ovo or vegan; CR: 5 male, 5 female; PL: 3 male, 5 female). Muscle biopsies (*vastus lateralis m*) were taken before and after the intervention and analyzed for IGF-I using standard immunohistochemical procedures. Stained muscle cross-sections were examined microscopically and IGF-I content quantified using image analysis software. Results showed that RET increased intramuscular IGF-I content by 67%, with greater accumulation from creatine supplementation (+78%) over placebo (+54%) (p=0.06). There were no differences in IGF-I between vegetarians and non-vegetarians. These findings indicate that creatine supplementation during resistance-exercise training increases intramuscular IGF-I concentration in healthy men and women, independent of habitual dietary routine.

P16
EFFECT OF CALORIC RESTRICTION ON ACYL COA OXIDASE AND LIPID PEROXIDES IN MICE.

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Aging related changes of lipid metabolism include a decline in peroxisome biosynthesis, lowered acyl CoA oxidase (AOX) activity, and accumulation of peroxides in tissues. Caloric restriction is known to mitigate age related changes in lipid metabolism. In this study we investigated the specific effect of caloric restriction (CR) on liver AOX, lipid peroxidation, and blood lipids. Mice were fed either ad libitum or a 30% calorically restricted diet for 12 months; liver and blood samples were obtained and analyzed for AOX, lipid peroxidation products, cholesterol, and triglyceride level. A significant increase in AOX was found

in liver as well as a reduction of lipid peroxidation products as assessed by TBAR assay. AOX activity was 1.0 nmol/min/mg protein in *ad libitum* fed mice and 1.9 nmol/min/mg protein in CR littermates; lipid peroxides also showed a decline in CR mice (1477 nmol MDA/mg liver protein) compared with *ad libitum* fed mice (1644 nmol MDA/mg liver protein). There were improvements of blood lipid profile as well in CR mice. Plasma cholesterol was 169 mg/dl in *ad libitum* fed mice and 50 mg/dl in CR mice; plasma triglycerides were 133 mg/dl and 144 mg/dl in CR and in *ad libitum* fed mice respectively. Our study demonstrates that CR is effective in increasing AOX, a rate limiting enzyme of β -oxidation in peroxisomes and reducing lipid peroxides in liver, changes that may reverse age related abnormalities of lipid metabolism.

P17
THE ASSOCIATION BETWEEN SELF-REPORTED DIETARY INTAKE AND INFLAMMATORY CYTOKINES IN OLDER ADULTS.

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Older adults have a greater risk of chronic disease due to an increased amount of inflammation. Dietary intake may influence the amount of inflammation especially in the elderly who may have compromised immune systems. The purpose of this research was to evaluate the association of self-reported dietary intake on the plasma concentration of IL-6 and TNF- α in elderly adults. **Methods:** We recruited 50 healthy older adults (65.4 \pm 5.9 yrs) from the general population by media advertisement to participate in this study. Self-reported dietary intake was assessed with a food frequency questionnaire (Block 98.2, Block Dietary Data Systems) modified for the typical Canadian diet. Plasma concentrations of IL-6 and TNF- α were assessed after a 12 hour overnight fast by ELISA. **Results:** Significant correlations existed between IL-6 and ω -3 fatty acid consumption ($r = -0.29$; $p \leq 0.05$), IL-6 and folate consumption ($r = -0.30$; $p \leq 0.05$), and TNF- α and saturated fat consumption ($r = 0.27$; $p \leq 0.05$). **Conclusion:** Decreased dietary intake of folate and ω -3 fatty acids is associated with increased IL-6 concentration while high dietary intake of saturated fat is associated with a high concentration of TNF- α in healthy older adults.

P18
OMEGA-3 SUPPLEMENTATION DOES NOT INCREASE KETONES IN PLASMA OF HEALTHY YOUNG AND ELDERLY ADULTS.

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Brain glucose uptake is impaired during healthy aging, an effect accentuated in people with cognitive decline. Ketones are alternative substrates which sustain brain energy supply in times of glucose shortage. The objective is to determine whether ketone production changes in the healthy elderly compared to young adults during a metabolic study day and following an omega-3 fatty acid supplementation. Subjects in two age groups (22 \pm 2 and 75 \pm 4 y old) were given a high fat meal after fasting for 12h. Plasma β -hydroxybutyrate (β -OHB), glucose, insulin, triacylglycerols, total cholesterol and free fatty acids (FFA) were measured during the subsequent 6 h before and following an omega-3 supplementation of 6 weeks (1.4 g/d of eicosapentaenoic acid (EPA) and 0.2 g/d of docosahexaenoic acid). Area under the curve (AUC) was calculated to estimate the metabolic response over the 6h. At baseline, EPA in the plasma of elderly was two fold higher compared to young. During omega-3 supplementation, EPA increased significantly by approximately 5 fold and FFA AUC was 20% lower in both groups, but FFA AUC in the elderly remained significantly higher compared to young adults, both before and after omega-3 supplementation. At baseline, plasma β -OHB AUC was similar between young and elderly but was 44% lower in young and 24% lower in elderly after omega-3 supplementation. FFA and β -OHB were significantly correlated before and after omega-3 supplementation in both age groups. However, for a similar concentration of plasma β -OHB in young and elderly, the corresponding concentration of FFA was higher in elderly but only before omega-3 supplementation. Omega-3 supplementation resulted in lower plasma FFA and β -OHB in both groups. These results suggest that before supplementation, the ability of the elderly to form β -OHB was lower compared to young adults.

Other Biological Fields

**P19
IMPAIRED HEPATIC INSULIN SENSITIZING
SUBSTANCE ACTION LEADS TO ADIPOSITY AND
DIABETES IN AGED RATS.**

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The hypotheses were: hepatic insulin sensitizing substance (HISS)-dependent insulin resistance (HDIR) accounts for insulin resistance that occurs with aging; HDIR is the initiating metabolic defect that leads progressively to type 2 diabetes and the metabolic syndrome; excess sucrose ingestion potentiates whereas a synergistic antioxidant cocktail in the chow attenuates this process. Male Sprague Dawley rats were tested at 9, 26 and 52 weeks to determine the dynamic response to insulin, the HISS-dependent component of insulin action and the HISS-independent (direct) insulin action using the rapid insulin sensitivity test. In the young rats, the HISS component accounted for $52.3 \pm 2.1\%$ of the response to a bolus of insulin (50 mU/kg) which decreased to $29.8 \pm 3.4\%$ at 6 months and $17.0 \pm 2.7\%$ at 12 months. Excess sucrose ingestion further decreased the HISS component to $18.3 \pm 3.4\%$ at 6 months and $6.2 \pm 1.5\%$ at 12 months. Aging was associated with generalized adiposity, higher plasma glucose, insulin and triglyceride, all of those changes being significantly potentiated by sucrose intake and strongly correlated with decreased HISS action ($r^2=0.67-0.87$). The changes in HISS action also correlated strongly with HOMA-IR and QUICKI indexes. The antioxidants (vitamin C, vitamin E, and S-adenosylmethionine) conferred protection of HISS action, adiposity, blood pressure, plasma insulin/glucose and triglyceride. Data are consistent with the hypotheses. Early detection and therapy directed towards treatment of HDIR offers a novel therapeutic target.

**P20
ROLE OF DIET IN TEENAGERS IN RELATION TO
CARDIOVASCULAR AND MOTOR FITNESS.**

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The study was conducted to explore the significance of diet (vegetarian and non vegetarian) along with the nutritional status on exercise performance of women athletes. One hundred and fifty women athletes (88 non vegetarians and 62 vegetarians) in the age group of 13-19 years attending national camps in preparation for international competition were included in the study. Anthropometric measurements viz; height, weight, body mass index were taken and dietary intake pattern was collected based on 24 hour recall. Cardiovascular Fitness was measured by Harvard Step Test. Motor Fitness parameters (balance, power, agility and speed) were measured by using appropriate tests. Blood samples were taken prior to exercise for assessment of hemoglobin. There was no significant difference between vegetarians and non vegetarians for energy intake. Protein intake was significantly higher in non vegetarians as compared to vegetarian and fat intake was higher in vegetarians as compared to non vegetarian group. Hemoglobin concentration was high in non vegetarians. Motor Fitness and recovery time after exercise i.e. Physical Efficiency Index (PEI) was better in non vegetarians than vegetarians. Endurance time was longer and recovery was faster in non vegetarian. Higher concentrations of hemoglobin in blood with its capacity to carry more oxygen may explain more endurance and better fitness in non vegetarians.

**P21
EFFECTS OF POWER TRAINING ON ANKLE
PLANTAR FLEXOR AND DORSIFLEXOR
STRENGTH AND POWER IN OLDER WOMEN.**

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Loss of muscle power with age is associated with declining function. The muscles around the ankle are important for mobility through their contributions to gait, balance and braking a vehicle. The objective of this study was to compare the effects of two high velocity concentric power training programs (weight machines and elastic bands) on ankle strength and power in mobility-impaired older women. Fifty women (70-88 years) with self-identified mobility limitations attended training sessions twice per week for 12 weeks after being randomized to one of three groups

(Weights, Bands, or Control). All subjects performed seated lower body exercises followed by power training for the dorsiflexor (DF) and plantar flexor (PF) muscles (Weights and Bands) or upper body flexibility exercises (Control). Ankle DF and PF strength and power capabilities were tested on an isokinetic dynamometer at 30°/s and 90°/s. DF strength and power improved significantly for all groups, with the greatest change occurring in the Weights group. PF strength increased by 17% in the Weights groups (30°/s). PF power improved at 30°/s and 90°/s for the Weights group (19% and 17% respectively) and at the higher velocity for the Bands group (12%). For this group of older women with mobility limitations, general lower extremity warm-up type exercises, elastic bands and weights were all similarly effective in improving DF strength and power. However, improvements in PF strength and power were largely limited to those who performed power training with weights.

P22
AGING ACCENTUATES THE DROP IN CEREBRAL BLOOD FLOW VOLUME BETWEEN SUPINE AND UPRIGHT POSTURES.

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Aging is associated with structural and functional changes to the vascular system that might influence cerebral autoregulation (CA). Intact CA implies that cerebral blood flow (CBF) is held constant in the face of changing pressure gradients across the cerebral vasculature, such as occurs on moving from bed to an upright posture. Transcranial Doppler ultrasound studies examining the middle cerebral artery have reported that healthy elderly individuals have intact CA; however, to date no study has compared quantitative CBF volume in supine and upright postures. The purpose of this study was to test the hypothesis that CBF volume will not change between supine and upright seated postures in young or healthy elderly individuals. A secondary hypothesis was that extreme, age-related structural and functional changes to central and peripheral vasculature impair the regulation of CBF volume between these postures. Extracranial CBF volume, common carotid intima-media thickness (IMT), brachial-ankle pulse wave velocity (baPWV), and common carotid distensibility (cDa) were examined in 34 young (aged 19-28) and 20 elderly (aged 67-92) individuals. Steady state CBF volume through the bilateral internal carotid and vertebral arteries was measured in both supine and seated postures. Interestingly, CBF volume was lower in the seated position than supine in both young and elderly groups (YOUNG – supine: 745 ± 98 vs. seated: 697 ± 35 mL/min, p = 0.001; ELDERLY – supine: 621 ± 51 vs. seated: 512 ± 25 mL/min, p = 0.0001). The absolute drop in brain blood flow was higher within the elderly group (YOUNG: 49 ± 35 vs. ELDERLY: 108 ± 30 mL/min, p = 0.001). Stratification of the elderly group by vascular risk factors further showed that individuals

in the quartile of highest risk for at least one of either IMT, baPWV or cDa had lower supine CBF volume than elderly individuals in the lower risk quartiles (HIGH RISK: 547 ± 105 vs. LOW RISK: 658 ± 55 mL/min, p = 0.04). The findings of this study demonstrate that quantitative CBF volume drops between supine and seated positions, notably even in young individuals when cerebral autoregulation would be expected to be functioning well. Also, elderly individuals with vascular risk factors have lower CBF volume while supine and are likely to be at a greater risk of under perfusion while in an upright posture.

P23
COLLAGEN 1A PROMOTER ACTIVITY IS INCREASED FOLLOWING MUSCLE CONTUSION IN OLD MICE.

Melanie Wilcox and Daniel Marsh, Department of Anatomy and Neurobiology, Dalhousie University. **Source of Research Funds:** NSERC.

The prevalence of fibrotic scarring after injury increases with age. Following muscle contusion, myogenic, fibrotic and adipogenic pathways are activated to varying degrees. We hypothesize that fibrosis progressively becomes the dominant pathway following injury, due to oxidative stress within the aging muscle. To test this hypothesis, we used ischemia/reperfusion to cause oxidative stress in muscle of young mice and compared contusion-induced fibrosis in ischemic muscle of young mice to muscle of aged mice. Subjects were transgenic C57BL/6 mice, containing the bacterial β-galactosidase (β-gal) *LacZ* gene, driven by the mouse *COL1A2* gene promoter. To induce ischemia, the anterior tibial artery was compressed for 2 h. Clip-contusion (50 g for 20 s) was performed upon the tibialis anterior muscle of 5, 15, and 75 week-old mice, with or without ischemia. Muscles were harvested at 7 days and 21 days post-injury, and expression of collagen was measured indirectly using a colorimetric assay for β-gal activity. Following contusion injury, levels of β-gal in muscle of old mice were higher than that detected in young tissue, indicating increased levels of fibrosis in aged muscle. When contusion was preceded by hypoxia in young muscle, fibrosis levels were comparable to that of old muscle. These results correspond with Western blots for collagen-1A. Western blots for myosin light chain indicate decreased myogenesis in old muscle following contusion injury, as compared to muscle of young mice. This study demonstrates that after muscle contusion, fibrosis levels increase with age, as a result of increased oxidative stress within the muscle. Increased fibrosis is observed in contused young muscle, when oxidative stress is induced by ischemia. Research supported by NSERC.

P25
FUNCTIONAL RESOLUTION OF FIBROSIS IN MDX MOUSE DYSTROPHIC HEART AND SKELETAL MUSCLE BY HALOFUGINONE.

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The effect of halofuginone (Halo) on established fibrosis in older mdx dystrophic muscle was investigated. Mice (8-9-months) treated with Halo (or saline in controls) for 5, 10 or 12 weeks were assessed weekly for grip strength and voluntary running. Echocardiography was performed at 0, 5, and 10 weeks. Respiratory function and exercise-induced muscle damage were tested. Heart, quadriceps, diaphragm and tibialis anterior muscles were collected to study fibrosis, collagen I and III expression, collagen content using a novel collagenase-digestion method, and cell proliferation. Hepatocyte growth factor (HGF) and alpha-smooth muscle actin (SMA) proteins were assayed in quadriceps. Halo decreased fibrosis (diaphragm and quadriceps), collagen I and III expression, collagen protein, and SMA content after 10 weeks treatment. Muscle-cell proliferation increased at 5 weeks, and HGF increased by 10 weeks treatment. Halo markedly improved both cardiac and respiratory function, and reduced damage and improved recovery from exercise. The overall impact of established dystrophy and dysfunction in cardiac and skeletal muscles was reduced by Halo treatment. Marked improvements in vital-organ functions implicate Halo as a strong candidate drug to reduce morbidity and mortality in DMD. (Supported by the Muscular Dystrophy Association, grant #3468, and a studentship from the Manitoba Institute of Child Health to KDH)

P26
DEVELOPMENT OF AN IN VIVO RAT MODEL FOR INDUCTION OF SPERMIDINE/SPERMINE N1-ACETYLTRANSFERASE.

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Amantadine is acetylated to N-acetylamantadine (ACA) solely by spermidine/spermine N1-acetyltransferase (SSAT1). Diethylnorspermine tetrahydrochloride (DENSPM) and alcohol are representative agents reported to induce SSAT activity in vitro. In five experimental series, rats were exposed to three concentrations of alcohol in their drinking water (5-15% v/v), DENSPM (50 mg/kg) alone, or DENSPM combined with alcohol. After each exposure amantadine HCl (3mg/kg) was injected i.p. and total urine was collected over 0-6 and 6-24 h. Volume and the pH of all urine samples were measured; then samples were frozen at -20C until analyzed for ACA by liquid chromatography/mass spectrometry. Pre- and post-

intervention controls were completed where rats drank water for one week and then were dosed with amantadine HCl to determine urine ACA. Control production of ACA was absent or present in trace amount in urine. Both alcohol and DENSPM induced SSAT1. Alcohol exposure increased urinary ACA with increasing concentration and duration of administration. ACA excretion in the 6-24 h urine was greater than in the 0-6 h sample. DENSPM was a more potent inducer than alcohol. Combined exposure to alcohol and DENSPM was synergistic for urinary ACA excretion. Longer exposure to alcohol combined with DENSPM administration provided the greatest potentiation of SSAT1 activity. This model will be valuable to determine the contribution of SSAT1 to acetylation of drugs administered as therapy in various disease states where SSAT1 is induced.

P27
CELLULAR MATRIX METALLOPROTEINASE SECRETION IS ALTERED BY CIGARETTE SMOKE EXTRACT.

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Gelatinases (MMP-2 and MMP-9) belong to a family of 26 cell-membrane bound or secreted, zinc-dependent proteolytic enzymes known as matrix metalloproteinases (MMPs). They are expressed in most mesenchymal cells. Gelatinases process a huge repertoire of extracellular molecules and degrade basement membrane laminins and type IV collagen. Therefore they play pivotal roles in a myriad of processes such as development, repair, regeneration and pathological conditions such as inflammation and cancers. Evidence suggests tobacco smoke exposure may alter release or function of MMPs in general and that of gelatinases in particular; these changes may relate to tobacco-induced diseases. The **Objective** was to examine and compare the effects of cigarette smoke extract on release of gelatinases by isolated cells related to primary sites of smoke exposure, the oral cavity and lung. **Methods:** rat periodontal ligament (rPDL) fibroblasts, fetal rat lung type II alveolar cells and fibroblasts were isolated following established protocols. Cells were exposed *in vitro* to increasing concentrations of cigarette smoke extract (CSE) with or without LPS. CSE was prepared by a modification of Carp and Janoff's method (*Carp and Janoff, 1978*) using 2R1 research cigarettes from Kentucky Tobacco Research Center, University of Louisville, USA. The conditioned media were collected and applied to 10% non-denatured gels impregnated with MMP-specific substrate (Gelatin A), employing a quantitative technique, gel zymography. This reveals specific molecular mass as well as bands of substrate degradation corresponding to MMP activity. Immunosorbent Enzyme-Linked Assays (ELISA Kits, R&D Systems) were

performed to identify and quantitate total MMP secretion. CSE-induced cellular and morphological changes were assessed by phase contrast microscopic imaging, while cellular proliferation and viability were examined by formazan and crystal violet assays. **Results:** showed that CSE with or without LPS altered significantly cellular MMP-2 and MMP-9 secretion in a concentration-dependent manner compared to values obtained from corresponding control groups. This increase was particularly marked in endodermally derived lung cells although under certain conditions periodontal ligament cells were also significantly reduced in proliferative capacity compared to the control samples. **Conclusions:** our studies suggest that CSE with or without LPS may alter viability and proliferation in rPDL cells, fetal rat lung type II cells and lung fibroblast and may modulate MMP gelatinase secretion. These changes may alter the rate of turnover of extracellular connective tissues. Thus local conditions may impact on matrix renewal rates, attachments, remodeling capacity or developmental interactions at the cellular level.

P28
BIPHASIC EFFECT OF OXLDL ON NUCLEAR PROTEIN IMPORT (NPI) AND CELL GROWTH IN VSMCS THROUGH MAPK ACTIVATION.

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OxLDL plays an important role in atherosclerosis partly by affecting cell proliferation and cell apoptosis. NPI is critical in regulating cell growth and proliferation. It is hypothesized that the mechanism whereby oxLDL affects proliferation and apoptosis is through an action on NPI. The aim of this study was to determine if exposure of aortic SMC to oxLDL can alter NPI, nuclear pore density, and cell growth through the MAPK pathway. Aortic SMC were exposed for different times to native LDL or oxLDL. Cells were then injected with a protein import substrate (Alexa 488-BSA-NLS) to visually monitor nuclear transport with the confocal microscope. The effect of MAPK inhibitors (SB203580 and PD98059) was investigated and western immunoblottings were also performed. Shorter exposure times of SMC to oxLDL significantly increased NPI, nuclear pore density (p62 expression) and expression of proliferation markers (PCNA) through an ERK MAPK-dependent mechanism. However, longer exposures to oxLDL depressed the rate and maximal NPI as well as nuclear pore density, and increased the expression of apoptosis markers (cleaved PARP) through a p38 MAPK-dependent mechanism. The data support the contention that the nucleus and NPI may represent a novel therapeutic target to control atherosclerosis.

P29
THE INDUCTION OF EARLY GROWTH RESPONSE 2 (Egr 2) IS TRIGGERED BY NEURONAL ACTIVITY DEPENDANT NF-κB ACTIVATION.

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NF-κB mediated signaling is complex and plays a critical role in many biological processes. Investigators have reported that NF-κB is activated during the induction of long term potentiation (LTP) and may be a requirement for synaptic plasticity and memory. In an attempt to explore target genes of NF-κB in LTP, we identified early growth response 2 (Egr 2) as one of targets of NF-κB. The present study focused on obtaining evidence of the linkage between the induction of Egr 2 and NF-κB signaling pathway. We analyzed transcriptomes of LTP-induced hippocampal slices (CA1 region) from 2-month-old NF-κB p50 knockout mice (p50^{-/-}) versus its littermate (p50^{+/+}) to identify target genes of NF-κB. LTP was evoked by applying theta-burst stimulation to Schaffer collateral axon in the CA1 region. At 3hr after conditioning, total mRNA samples were extracted from LTP-induced slices and non-stimulated control slices. These mRNA samples were subjected to the DNA microarray analysis (Affymetrix GeneChip[®] Mouse Genome 430 2.0) and real-time RT-qPCR. We also examined the mRNA and protein expression level of Egr 2 in HeLa cells using real-time RT-qPCR and Western blotting. TNF α was used for activating NF-κB signaling pathway in HeLa cells. The P-Match software was used for sequence analysis of distal promoter region of Egr 2. There were no significant differences of both basal synaptic transmission and LTP magnitude in p50^{-/-} and p50^{+/+}. We identified early growth response 2 (Egr2) is induced by NF-κB activation during LTP. From the gene-structure analysis, we found several NF-κB consensus binding sites around promoter region of Egr2. In addition, the upregulation of Egr2 mRNA in HeLa cells treated with TNFα, an activator of the NF-κB signaling pathway, has been observed. These data suggest that Egr2 expression level is controlled by direct transcriptional activity of NF-κB.